STATEMENT

I, Hironori KATSURA—of Toranomon East Bldg. 7-13, Nishi-Shimbashi 1-chome, Minato-ku, Tokyo 105-8408 Japan—hereby declare that I am conversant in both Japanese and English and that I believe the following is a true and correct translation of a copy of Japanese Patent Application No. 2003-317104.

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(General Power of attorney No.)	0205141	

[Designation of Document] SCOPE OF CLAIMS

[Claim 1]

A method for isolating and purifying a nucleic acid, comprising the step of:

adsorbing nucleic acid onto a solid phase and desorbing nucleic acid from the solid phase, using a solution capable of adsorbing nucleic acid onto a solid phase and a solution capable of desorbing nucleic acid from the solid phase, respectively;

wherein the solution capable of adsorbing nucleic acid contains an antifoaming agent.

[Claim 2]

The method for isolating and purifying a nucleic acid according to claim 1, wherein the solid phase is a solid phase comprising an organic polymer having hydroxyl groups on the surface.

[Claim 3]

The method for isolating and purifying a nucleic acid according to claim 1, wherein the solid phase is a solid phase comprising silica or derivative thereof, diatomaceous earth or alumina.

[Claim 4]

The method for isolating and purifying a nucleic acid according to claim 2, wherein the organic polymer having hydroxyl groups on the surface is a saponified product of

acetyl cellulose.

[Claim 5]

The method for isolating and purifying a nucleic acid according to claim 4, wherein the organic polymer having hydroxyl groups on the surface is a saponified product of triacetyl cellulose.

[Claim 6]

The method for isolating and purifying a nucleic acid according to claim 5, wherein the acetyl cellulose is a porous membrane.

[Claim 7]

The method for isolating and purifying a nucleic acid according to any one of claims 1 to 6, wherein the antifoaming agent further contains two components of silicon type antifoaming agent and alcohol type antifoaming agent.

[Claim 8]

The method for isolating and purifying a nucleic acid according to claim 7, wherein the alcohol type antifoaming agent is an acethylene glycol type surface-active agent.

[Claim 9]

The method for isolating and purifying a nucleic acid according to any one of claims 1 to 8, wherein the adsorption and desorption of nucleic acid are carried out using a unit for isolating and purifying a nucleic acid,

which houses the solid phase in a container having at least two openings.

[Claim 10]

The method for isolating and purifying a nucleic acid according to any one of claims 1 to 9, wherein the adsorption and desorption of nucleic acid are carried out using a unit for isolating and purifying a nucleic acid, which comprises:

(a) the solid phase;

(b) a container having at least two openings, which houses the solid phase; and

(c) an apparatus for generating the pressure difference, which is connected to one of the openings of the container.

[Claim 11]

The method for isolating and purifying a nucleic acid according to claim 10, wherein the apparatus for generating the pressure difference is an apparatus for pressurization.

[Claim 12]

The method for isolating and purifying a nucleic acid according to claim 10, wherein the apparatus for generating the pressure difference is an apparatus for pressure reduction.

[Claim 13]

A solution for adsorbing nucleic acid onto the solid

phase used in the method for isolating and purifying a nucleic acid according to any one of claims 1 to 12, the solution comprising an antifoaming agent.

[Designation of Document] SPECIFICATION

[Title of the Invention] METHOD FOR ISOLATAING AND PURIFYING NUCLEIC ACID

[Technical Field]

[0001]

The present invention relates to a method for isolating and purifying a nucleic acid, and a solution used in the method for adsorbing nucleic acid onto a solid phase.

[Background Art]

[0002]

Nucleic acid has been used in various forms in various fields. In the field of recombinant nucleic acid art, for example, it is demanded that nucleic acid is used in forms of probe, genomic nucleic acid and plasmid nucleic acid.

[0003]

Nucleic acid is used in various forms for various objects in the field of diagnosis as well. For example, nucleic acid probe is routinely used for detection and diagnosis of human pathogen. Similarly, nucleic acid is used for detection of genetic disorder and detection of food-polluting substances as well. Further, nucleic acid is routinely used in confirmation of position, identification and isolation of predetermined nucleic acid for various purposes such as preparation of genetic map,

cloning and gene expression by genetic recombination.

[0004]

In many cases however, nucleic acid is available only in a very limited amount and its operation for isolation and purification is troublesome and much time is needed therefor. Such a time-consuming troublesome step has a problem that it is apt to result in loss of nucleic acid. In addition, in the case of purification of nucleic acid from a sample obtained from serum, urine and culture of bacteria, there is a problem that contamination is generated resulting in a pseudo-positive result.

[0005]

As one of the methods for easily and efficiently isolating and purifying a nucleic acid, there disclosed a method using a solution in which nucleic acid is adsorbed with a solid phase and a solution in which nucleic acid is desorbed from the solid phase, in which nucleic acid is adsorbed and desorbed, respectively, with and from the solid phase comprising an organic polymer having hydroxyl groups on the surface, whereby nucleic acid is isolated and purified (Japanese Patent Laid-Open No. 2003-128691).

[0006]

[Patent Document 1] Japanese Patent Laid-Open No. 2003-128691

[Disclosure of the Invention]

[Problem that the Invention is to Solve]

[0007]

In conducting the method for isolating and purifying a nucleic acid as such and particularly in using whole blood as a sample, there is a problem that a solution obtained from the sample becomes highly viscous. Further, in adding a surface-active agent for dissolving the cell membrane of the cells in the sample, there is a problem that, in a step of adsorption of nucleic acid with a solid phase, bubbling becomes vigorous as a result of a secondary effect of the surface-active agent. Because of the contamination by scattering of the foams and the influence on the time required for isolating and purifying a nucleic acid, there has been a demand for suppressing the generation of foams. Accordingly, an object of the present invention is to provide a method for isolating and purifying a nucleic acid, comprising adsorption of nucleic acid onto a solid phase, washing the solid phase under such a state that nucleic acid is adsorbed therewith and desorbing nucleic acid from the solid phase, in which foams generated during the steps are able to be eliminated.

[Means for Solving the Problem]

[0008]

The present inventors have keenly studied to solve the above problems, and as the result, they have found that

when a solution for adsorbing nucleic acid to a solid phase contains an antifoaming agent, generation of foams is able to be suppressed. It has been particularly found in the present invention that, when the solution contains two components of silicon type antifoaming agent and alcohol type antifoaming agent, generation of foams can be dramatically suppressed. The present invention has been achieved on the basis of those findings.

[0009]

That is, the present invention provides a method for isolating and purifying a nucleic acid, including the step of adsorbing nucleic acid onto a solid phase, and desorbing nucleic acid from the solid phase, using a solution capable of adsorbing nucleic acid onto a solid phase and a solution capable of desorbing nucleic acid from a solid phase, respectively, in which the solution capable of adsorbing nucleic acid contains an antifoaming agent.

[0010]

It is preferable that the solid phase is a solid phase comprising an organic polymer having hydroxyl groups on the surface, or a solid phase comprising silica or derivative thereof, diatomaceous earth or alumina.

It is preferably that the organic polymer having hydroxyl group on the surface is a saponified product of acetyl cellulose.

It is more preferable that the organic polymer having hydroxyl group on the surface is a saponified product of triacetyl cellulose.

It is preferable that the acetyl cellulose is a porous membrane.

[0011]

It is preferable that the antifoaming agent contains two components of silicon type antifoaming agent and alcohol type antifoaming agent.

It is more preferable that the alcohol type antifoaming agent is an acethylene glycol type surface-active agent.

[0012]

It is preferable that the adsorption and desorption of nucleic acid are carried out using a unit for isolating and purifying a nucleic acid, which houses the solid phase in a container having at least two openings.

It is more preferable that the adsorption and desorption of nucleic acid are carried out using a unit for isolating and purifying a nucleic acid, which comprises (a) the solid phase, (b) a container having at least two openings, which houses the solid phase, and (c) an apparatus for generating the pressure difference, which is connected to one of the openings of the container. The apparatus for generating the pressure difference may be

either an apparatus for pressurization or an apparatus for pressure reduction.

[0013]

According to another aspect of the invention, there provided a solution capable of adsorbing nucleic acid onto the solid phase used in the method for isolating and purifying a nucleic acid, which contains an antifoaming agent.

[Advantage of the Invention]

[0014]

According to the invention, generation of foams is able to be suppressed, thus nucleic acid having high purity can be efficiently separated from a sample solution containing nucleic acid.

[Best Mode for Carrying out the Invention]

[0015]

Hereinafter, the mode of the invention will be explained.

The method for isolating and purifying a nucleic acid of the invention relates to a method for isolating and purifying a nucleic acid from a sample containing nucleic acids, where the method includes the steps of adsorbing nucleic acid in the sample onto a solid phase (for example, a solid phase comprising an organic polymer having hydroxyl groups on the surface), and desorbing nucleic acid from the

solid phase, in which the solution capable of adsorbing nucleic acid contains an antifoaming agent.

[0016]

Specific examples of the antifoaming agent include an antifoaming agent of a silicon type (such as silicone oil, dimethylpolysiloxane, silicone emulsion, modified polysiloxane and silicone compound), an antifoaming agent of an alcohol type (such as acetylene glycol, heptanol, ethylhexanol, higher alcohol and polyoxyalkylene glycol), an antifoaming agent of an ether type (such as heptyl cellosolve and nonyl cellosolve-3-heptylcorbitol), an antifoaming agent of a fat type (such as animal and plant oil), an antifoaming agent of a fatty acid type (such as stearic acid, oleic acid and palmitic acid), an antifoaming agent of a metal soap type (such as aluminum stearate and calcium stearate), an antifoaming agent of a fatty acid ester type (such as natural wax and tributyl phosphate), an antifoaming agent of a phosphate type (such as sodium octylphosphate), an antifoaming agent of an amine type (such as diamylamine), an antifoaming agent of an amide type (such as stearic amide) and other antifoaming agents (such as ferric sulfate and bauxite). Particularly preferably, two of silicon type and alcohol type antifoaming agents may be used in combination as an antifoaming agent. With regard to the antifoaming agent of

an alcohol type, surface-active agent of an acetylene glycol type is preferred.

[0017]

In the present invention, "nucleic acid" may be any of single-stranded and double-stranded one. There is no limitation for the molecular weight thereof as well.

[0018]

"Sample" means any sample containing nucleic acid. Type of the nucleic acid in the sample solution may be either one or more. With regard to the length of each nucleic acid, there is no particular limitation and, for example, it may be a nucleic acid having any length from several bp to several Mbp. In view of handling, it is usually preferred that the length of nucleic acid is from about several bp to several hundred kbp.

[0019]

With regard to a solid phase, there is no particular limitation and there may be used, for example, a solid phase comprising an organic polymer having hydroxyl group on the surface, a solid phase comprising silicon dioxide, silica polymer or magnesium silicate or a solid phase comprising silica or derivative thereof, diatomaceous earth or alumina. A solid phase comprising an organic polymer having hydroxyl groups on the surface may be preferably used.

[0020]

As an organic polymer having hydroxyl groups on the surface, a saponified product of acetyl cellulose is preferable. Acetyl cellulose may be any one of monoacetyl cellulose, diacetyl cellulose, and triacetyl cellulose, but triacetyl cellulose is particularly preferable. It is preferable that in the invention, acetyl cellulose of which the surface is saponified is used as a solid phase. Herein, "surface saponified" means that only the surface in contact with a saponification treatment liquid (for example, NaOH) is saponified. In the invention, it is preferable that the structure of the solid phase is acetyl cellulose and only the surface of the solid is saponified. Accordingly, the amount (density) of hydroxyl groups in the solid surface can be controlled by the degree of the saponification treatment (saponification degree).

[0021]

It is preferable that an organic polymer having hydroxyl groups on the surface is prepared in the form of a membrane, in order to enlarge a surface area of an organic polymer having hydroxyl groups on the surface. In addition, acetyl cellulose may be either a porous membrane or a nonporous membrane, but it is more preferable that the membrane is porous. It is preferable that when the solid phase is a porous membrane, the structure of the membrane

is acetyl cellulose as it is, and only the surface of the solid is saponified. Accordingly, the spatial amount (density) of hydroxyl groups can be controlled by the degree of the saponification treatment (saponification degree) × pore diameter. In addition, the structure of the membrane is constituted by acetyl cellulose, thereby obtaining a strong solid phase.

[0022]

For example, the membrane of triacetyl cellulose is commercially available from Fuji Photo Film Co., Ltd. under a trade name of TAC base, and the porous membrane of triacetyl cellulose is available as microfilter FM500 (manufactured by Fuji Photo Film Co., Ltd.).

In addition, it is also preferable that, for example, membrane of triacetylcellulose may be formed on the surface of beads made of polyethylene, and saponified to contain a hydroxyl group on the surface. In this case, triacetylcellulose is coated on the beads. Material for the beads is not limited to polyethylene but may be anything so far as it does not, for example, pollute nucleic acid.

[0023]

It is preferable that the number of hydroxyl groups is large in order to improve a separating efficiency of the nucleic acid. For example, in the case of using acetyl

cellulose such as triacetyl cellulose, a saponification rate is preferably about 5% or more, and more preferably 10% or more.

For saponifying acetyl cellulose, a sample to be saponified is dipped in an aqueous solution of sodium hydroxide. In order to change the saponification rate, the concentration of sodium hydroxide is changed. The saponification rate is deteramined by quantitating a residual acetyl group by NMR.

[0024]

In the method for isolating and purifying a nucleic acid according to the present invention, adsorption and desorption of nucleic acid is able to be carried out using a cartridge for isolating and purifying a nucleic acid where the above-mentioned solid phase (for example, a solid phase comprising an organic polymer having hydroxyl groups on the surface) is received in a container preferably having at least two openings.

[0025]

More preferably, a method for isolating and purifying a nucleic acid according to the present invention uses a unit for isolating and purifying a nucleic acid containing

(a) a solid phase(for example, a solid phase comprising an organic polymer having hydroxyl groups on the surface),

(b) a container having at least two openings which receives the above solid phase and

(c) an apparatus for generating the pressure difference connected to one of the openings of the above container.

[0026]

The first embodiment of the method for isolating and purifying a nucleic acid according to the present invention may include the following steps:

(a) a step where one of openings of the unit for isolating and purifying a nucleic acid is inserted into the sample solution containing nucleic acid,

(b) a step where the sample solution containing nucleic acid is sucked when the inner area of the container is made in a state of reduced pressure using an apparatus for generation of pressure difference connected to another opening of the unit for isolating and purifying a nucleic acid and then it is contacted to the solid phase,

(c) a step where the inner area of the container is made into a pressurized state using an apparatus for generation of pressure difference connected to another opening of the unit for isolating and purifying a nucleic acid, and the sucked sample solution containing nucleic acid is discharged outside the container,

(d) a step where one opening of the unit for

isolating and purifying a nucleic acid is inserted into a buffer solution for washing nucleic acid,

(e) a step where the nucleic acid washing buffer solution is sucked when the inner area of the container is made in a state of reduced pressure using an apparatus for generation of pressure difference connected to another opening of the unit for isolating and purifying a nucleic acid and then it is contacted to the solid phase,

(f) a step where the inner area of the container is made into a pressurized state using an apparatus for generation of pressure difference connected to another opening of the unit for isolating and purifying a nucleic acid and the sucked nucleic acid washing buffer solution is discharged outside the container,

(g) a step where one opening of the unit for isolating and purifying a nucleic acid is inserted into a solution for desorbing the nucleic acid adsorbed onto the solid phase,

(h) a step where the solution capable of desorbing nucleic acid which is adsorbed in the solid phase is sucked when the inner area of the container is made in a state of reduced pressure using an apparatus for generation of pressure difference connected to another opening of the unit for isolating and purifying a nucleic acid and then it is contacted to the solid phase and

(i) a step where the inner area of the container is made into a pressurized state using an apparatus for generation of pressure difference connected to another opening of the unit for isolating and purifying a nucleic acid and the sucked elution solution is discharged outside the container.

[0027]

The second embodiment of the method for isolating and purifying a nucleic acid according to the present invention may include the following steps:

(a) a step of preparing a sample solution containing nucleic acid from a sample and injecting the sample solution containing nucleic acid into one of the openings of the unit for isolating and purifying a nucleic acid;

(b) a step of making the inner area of the container into a pressurized state by using the apparatus for generating the pressure difference being connected to the one of the openings of the unit for isolating and purifying a nucleic acid and contacting the injected sample solution containing nucleic acid to the solid phase by discharging the sample solution from another opening of the container;

(c) a step of injecting nucleic acid washing buffer into one of the openings of the unit for isolating and purifying a nucleic acid;

(d) a step of making the inner area of the container

into a pressurized state by using the apparatus for generating the pressure difference being connected to one of the openings of the unit for isolating and purifying a nucleic acid and discharging the injected nucleic acid washing buffer from another opening of the container to contact the nucleic acid washing buffer to the solid phase;

(e) a step of injecting a solution capable of desorbing nucleic acid which is adsorbed in the solid phase into one of the openings of the unit for isolating and purifying a nucleic acid; and

(f) a step of making the inner area of the container into a pressurized state by using the apparatus for generating the pressure difference being connected to one of the openings of the unit for isolating and purifying a nucleic acid and discharging the injected solution capable of desorbing nucleic acid from another opening of the container to desorb the adsorbed nucleic acid from the solid phase and discharge nucleic acid outside the container.

[0028]

The method for isolating and purifying nucleic acid according to the invention will be further explained in detail. In the invention, it is preferable that a sample solution containing nucleic acid is brought into contact with a solid phase (for example, a solid phase comprising

an organic polymer having hydroxyl groups on the surface) to adsorb nucleic acid in the sample solution onto the solid phase, and then the nucleic acid adsorbed onto solid phase is desorbed from the solid phase using a suitable solution explained as follows. It is more preferable that the sample solution containing nucleic acid is a solution prepared by adding a water-soluble organic solvent to a solution in which nucleic acid is dispersed therein by treating a sample containing cells or viruses with a solution which dissolves cell membrane and nuclear membrane, and then further adding an antifoaming agent.

[0029]

With regard to the sample solution containing nucleic acid which is able to be used in the present invention, there is no particular limitation, but in the diagnostic field for example, body fluid which is collected as a sample such as whole blood, plasma, serum, urine, feces, sperm and saliva, or plant (or a part thereof), animal (or a part thereof), or lysate thereof and a solution prepared from a biological material such as homogenate may be used.

[0030]

Firstly, these samples are treated with an aqueous solution containing a reagent which dissolves cell membrane and solubilizes nucleic acid. Accordingly, the cell membrane and nuclear membrane are dissolved, thereby

dispersing nucleic acid into the aqueous solution.

[0031]

For the dissolution of a cell membrane and the solubilization of nucleic acid, there required, for example, (1) removal of red blood corpuscles, (2) removal of various proteins and (3) dissolving of white blood corpuscles and dissolving of nuclear membrane, when the sample which is an object is whole blood for example. (1) removal of red blood corpuscles and (2) removal of various proteins, and (3) dissolving of white blood corpuscles and dissolving of nuclear membrane, when the sample which is an object is whole blood for example are necessary for the purpose of preventing a non-specific absorption to the solid phase and a clogging of a porous membrane, and for the purpose of the solubilization of nucleic acid which is an object of the extraction, respectively. In particular, (3) dissolving of white blood corpuscles and dissolving of nuclear membrane is important process, and the solubilization of nucleic acid in the process are necessary in the invention. It is possible to achieve the above-mentioned (1), (2) and (3) at the same time by, for example, incubating at 60°C for 10 minutes after addition of guanidine hydrochloride, Triton-X 100 and protease K (manufactured by Sigma).

[0032]

The reagent for solubilization of nucleic acid used

in the invention may be exemplified by guanidine salt, surface-active agent, protease, or antifoaming agent.

[0033]

As guanidine salt, guanidine hydrochloride is preferable, but the other guanidine salt (guanidine isothiocyanate and guanidine thiocyanate) may be used. The concentration of guanidine salt in the solution is 0.5 M or more to 6 M or less, and preferably 1 M or more to 5 or less.

[0034]

As the surface-active agent, Triton-X100 may be used, in addition to this, an anionic surface-active agent such as SDS, sodium cholate, and sarcosine sodium; a nonionic surface-active agent such as Tween 20 and megafack; and other various amphoteric surface-active agents may be used. A nonionic surface-active agent such as polyoxyethylene octyl phenyl ether (Triton-X100) is preferably used in the invention. The concentration of the surface-active agent in the solution is generally 0.05 % by weight to 10 % by weight, preferably 0.1% by weight to 5 % by weight.

[0035]

As protease, protease K may be used, but the same effect can be also obtained by using other protease. Since protease is an enzyme, protease is preferably used while being heated, preferably at 37°C to 70°C, and particularly

preferably at 50°C to 65°C.

[0036]

As the antifoaming agent, those described in the specification may be used.

[0037]

As described above, a water-soluble organic solvent is added to an aqueous solution in which nucleic acid is dispersed so as to allow the contact with the solid phase. By this procedure, nucleic acid in the sample solution is adsorbed onto the solid phase (for example, a solid phase comprising an organic polymer having hydroxyl groups on the surface). In order to allow the nucleic acid solubilized by the above described procedure in the specification to be adsorbed onto the solid phase comprising an organic polymer having hydroxyl groups on the surface, it is necessary that a water-soluble organic solvent is mixed with a mixed solubilized nucleic acid solution and a salt is present in the resulting solution of mixed nucleic acid.

[0038]

Accordingly, hydrated structure of water molecules existing around nucleic acid is destroyed and nucleic acid is solubilized in an unstable state. It is believed that, when nucleic acid under such a state is contacted to a solid phase comprising an organic polymer having hydroxyl groups on the surface, an interaction takes place between

polar groups on the surface of nucleic acid and polar groups on the surface of solid phase whereby nucleic acid is adsorbed with the surface of the solid phase. According to the present invention, nucleic acid is able to be made into an unstable state by the fact that a water-soluble organic solvent is mixed with a solution of mixed solubilized nucleic acid solution and that a salt is present in the resulting solution of mixed nucleic acid.

[0039]

Examples of the water-soluble organic solvent used herein include ethanol, isopropanol and propanol. Among them, ethanol is preferred. The concentration of the water-soluble organic solvent is preferably 5 % by weight to 90% by weight, more preferably 20 % by weight to 60% by weight. With regard to the concentration of ethanol added thereto, it is particularly preferred to be as high as possible within the above range so far as no aggregate is produced.

[0040]

Examples of the salt which is present in the resulting solution of mixed nucleic acid, are preferably various chaotropic substances (such as guanidium salt, sodium iodide and sodium perchlorate), sodium chloride, potassium chloride, ammonium chloride, sodium bromide, potassium bromide, calcium bromide and ammonium bromide.

Guanidium salt is particularly preferred since it has both effects of solubilization of cell membrane and solubilization of nucleic acid.

[0041]

Next, the solid phase onto which nucleic acid is adsorbed is brought into contact with nucleic acid washing buffer solution. The solution has a function of washing out impurities in the sample solution which is adsorbed with the solid phase together with nucleic acid. For such a purpose, it is necessary that the solution has a composition capable of not desorbing nucleic acid from the solid phase, but desorbing the impurity from the solid phase. The nucleic acid washing buffer solution comprises a base compound and buffer, and an aqueous solution containing a surface-active agent if necessary. The base compound may be exemplified by approximately 10 to 100 % by weight (preferably approximately 20 to 100 % by weight, and more preferably approximately 40 to 80 % by weight) of an aqueous solution of methanol, ethanol, isopropanol, n-isopropanol, butanol, acetone or the like. With regard to buffer and surface-active agent, those which are already mentioned may be exemplified. Among them, a solution containing ethanol, Tris and Triton-X100 is preferable. Preferred concentrations of Tris and Triton-X100 are 10 to 100 mM and 0.1 to 10% by weight, respectively.

[0042]

Next, the washed solid is brought into contact with the solution capable of desorbing nucleic acid adsorbed onto the solid phase. Since the solution contains a target nucleic acid, the solution is recovered to be used in the next procedure, for example, an amplification of nucleic acid with PCR (polymerase chain reaction). It is preferable that the solution capable of desorbing nucleic acid has a low concentration of salt. It is particularly preferably that the solution having the concentration of salt of 0.5 M or less is used. As such solution, purified distilled water, TE buffer or the like may be used.

[0043]

The unit for isolating and purifying a nucleic acid used in the invention is a unit containing a solid phase comprising an organic polymer having hydroxyl groups on the surface, a container having at least two openings which receives the above solid phase.

There is no particular limitation for a material of the container and anything may be used so far as it is able to receive an organic polymer having hydroxyl groups on the surface and is able to install at least two openings. In view of easiness in the manufacture, plastic is preferred. It is preferred to use a transparent or opaque plastic such as polystyrene, polymethacrylic ester, polyethylene,

polypropylene, polyester, Nylon and polycarbonate.

[0044]

The container may be in such a state that it has a receiving part for a solid phase, that a solid phase is able to be received in the receiving part, that the solid phase does not come out of the receiving part upon suction and discharge of the sample solution, etc. and that an apparatus for generating the pressure difference, for example, a syringe is able to be connected to an opening. For such a purpose, it is preferred that the container is initially divided into two parts and, after receiving the solid phase, they are able to be united. In addition, it is possible to place meshes prepared by a material which does not pollute DNA on and beneath the solid phase, so that coming-out of the solid phase from the receiving part is avoided.

[0045]

There is also no particular limitation for the shape of a solid phase received in the above-mentioned container and that may be any shape such as circular, square, rectangular and elliptic and, in the case of membrane, it may be tubular, rolled or beady where an organic polymer having hydroxyl group on the surface is coated. In view of a manufacturing aptitude, a shape having high symmetry such as circle, square, cylinder or roll and beads are preferred.

[0046]

One of the openings of the container is inserted into the sample solution containing nucleic acid, sucked from the other opening, the sample solution is brought into contact with a solid phase comprising an organic polymer having hydroxyl groups on the surface and discharged, subsequently, a nucleic acid washing buffer solution is sacked and discharged, then the solution capable of desorbing nucleic acid which is adsorbed in an organic polymer having hydroxyl groups on the surface is sacked and discharged, and the discharged solution is recovered, thereby obtaining a target nucleic.

[0047]

The unit for isolating and purifying a nucleic acid used in the invention preferably contains:

(a) a solid phase(for example, a solid phase comprising an organic polymer having hydroxyl groups on the surface),

(b) a container having at least two openings which receives the above solid phase and

(c) an apparatus for generating the pressure difference connected to one of the openings of the above container. The unit for isolating and purifying nucleic acid will be explained as follows.

[0048]

The container is usually prepared in a manner of being divided into a body receiving the solid phase and a cover where at least one opening is installed in each of them. The opening is used as an inlet and outlet for a sample solution containing nucleic acid, a nucleic acid washing buffer solution and an solution capable of desorbing nucleic acid which is adsorbed in the solid phase (hereinafter, they will be referred to as "sample solution, etc.") and is connected to an apparatus for generation of pressure difference by which the inner area of the container is able to be made into a state of reduced or high pressure. Although there is no particular limitation for the shape of the body, it is preferred that its cross section is made circular whereby the manufacture is easy and the sample solution, etc. are apt to be diffused on the whole surface of the solid state. It is also preferred to make the cross section square so that no offcut of the solid phase is generated.

[0049]

It is necessary that the above cover is connected to the body in such a manner that the inner area of the container is made into a state of reduced or high pressure by means of the apparatus for generating the pressure difference and, when once such a state is achieved, the connecting method is able to be freely selected. Examples

thereof are use of an adhesive, stuffing, inserting, screwing and fusion by means of ultrasonic heating.

[0050]

It is preferred that inner volume of a container is determined by only the volume of a sample solution to be treated and, usually, it is expressed in terms of volume of a solid phase to be received therein. Thus, it is preferred to be in such a size that one to about six sheet(s) of solid phase where thickness is about 1 mm or less (such as about 50 to 500 μm) and diameter is about 2 mm to 20 mm is/are received.

[0051]

It is preferred that the end of the side contacting the container of the solid phase is closely adhered to the inner wall surface of the container to such an extent that a sample solution, etc. do not pass.

[0052]

It is preferred that the beneath the opening used as an inlet for a sample solution, etc. from the solid phase is made in such a structure that it not closely adhered to the inner wall of the container but space is placed there so that a sample solution, etc. is diffused to whole surface of the solid phase as uniform as possible.

[0053]

It is preferred to install a material where pore is

formed near its center on a solid phase encountering the opening connected to the apparatus for generation of the pressure difference. The material has an effect of pressing down the solid phase and also discharging the sample solution, etc. efficiently and it is preferred in a shape of having a slope such as funnel or cup so that the solution is accumulated to the central pore. Size of the pore, angle of the slope and thickness of the material are able to be appropriately determined by persons skilled in the art by taking the amount of the sample solution, etc. to be treated, the size of the container receiving the solid phase and so on into consideration. It is preferred that, between the material and the opening, a space for storing the overflowed sample solution, etc. and for prevention of sucking into the apparatus for generating the pressure difference is installed. Size of the space is also able to be appropriately selected by persons skilled in the art. Incidentally, for a purpose of efficient collection of nucleic acid, it is preferred to suck a sample solution containing nucleic acid in an amount which is not less than that whereinto the entire solid phase is dipped.

[0054]

In addition, for such a purpose that concentration of the sample solution, etc. only to the part immediately

beneath the sucking opening so that the sample solution, etc. are able to pass through the solid phase relatively uniformly, it is preferred that a space is installed between the solid phase and the material as well. For such a purpose, it is preferred to install plural projections from the material to the solid phase. Although size and numbers of the projections may be appropriately selected by persons skilled in the art, it is preferred that they are as large as possible within such an extent that the opening area of the solid phase is able to be kept as big as possible while the space is retained.

[0055]

When three or more openings are installed in a container, it goes without saying that an excessive opening is to be temporarily closed so that suction and discharge as a result of an operation for giving reducing and high pressure are made possible.

[0056]

By means of an apparatus for generating the pressure difference, firstly, the inner area of the container housing a solid phase is made into a state of reduced pressure and then the sample solution containing nucleic acid is sucked. Examples of an apparatus for generation of pressure difference are injection syringe, pipetter and pump which is able to suck and compress such as peristaltic

pump. Among them, injection syringe and pump are suitable for manual operation and automatic operation, respectively. Pipetter has an advantage that an operation by one hand is easily carried out. Preferably, the apparatus for generation of pressure difference is connected in a detachable manner to one opening of the above-mentioned container.

[0057]

Next, the method for purifying nucleic acid using the unit for isolating and purifying nucleic acid will be explained. Firstly, one of openings of the unit for isolating and purifying a nucleic acid is inserted into the sample solution containing nucleic acid. Next, the sample solution containing nucleic acid is sucked into the container when the inner area of the container is made into a state of reduced pressure using an apparatus for generation of pressure difference connected to one of the other openings. By the procedure, the sample solution is contacted with the solid phase, and thus nucleic acid in the sample solution is adsorbed onto the solid phase. At this time, it is preferred that a sample solution is sucked in such an amount that it contacts to nearly the whole area of the solid phase. However, the apparatus is polluted when it is sucked into an apparatus for generating the pressure difference and, therefore, that is appropriately

adjusted.

[0058]

After an appropriate amount of a solution is sucked, the inner area of the container is pressurized using the apparatus for generating the pressure difference and the sucked solution is discharged. It is not necessary to make time until such an operation but a discharge may be conducted immediately after the suction.

[0059]

Next, the nucleic acid washing buffer solution is sacked into the container by the same depressurization and pressurization as described above and discharged, and an inner container is washed. The solution has a function of washing out impurities in the sample solution which is adsorbed with the solid phase together with nucleic acid, as well as the residual sample solution in the container. For such a purpose, it is necessary that the solution has a composition capable of not desorbing nucleic acid from the solid phase, but desorbing the impurity from the solid phase. The nucleic acid washing buffer solution comprises a base compound and buffer, and an aqueous solution containing a surface-active agent if necessary. The base compound may be exemplified by approximately 10 to 90% (preferably approximately 50 to 90%) of an aqueous solution of methyl alcohol, ethyl alcohol, butyl alcohol, acetone or

the like. With regard to buffer and surface-active agent, those which are already mentioned may be exemplified. Among them, a solution containing ethyl alcohol, Tris and Triton-X100 is preferable. Preferred concentrations of Tris and Triton-X100 are 10 to 100 mM and 0.1 to 10%, respectively.

[0060]

Next, the solution capable of desorbing nucleic acid which is adsorbed in the solid phase is introduced in the container, and discharged outside the container. As the discharged solution contains nucleic acid which is target, the discharged solution is recovered and subjected to the next procedure such as an amplification of nucleic acid due to PCR (polymerase chain reaction).

The present invention will now be further illustrated by way of the following Examples although the present invention is not limited thereto.

[Examples]

[0061]

(1) Materials and reagents

Using a cartridge for purifying nucleic acid having a structure as shown in Fig. 1 to Fig. 6, a sample, washing solution and distilled water were successively injected from the second opening side and, for each operation, a piston material (plunger) is inserted and pushed. With

regard to a solid phase for adsorption of nucleic acid, Fuji Microfilter FR 250 (manufactured by Fuji Photo Film) was used. An adsorption buffer solution for purifying nucleic acid (Comparative Example and the present invention) and washing buffer solution were prepared as follows.

[0062]

Adsorption buffer (Comparative Example)

Guanidine hydrochloride (manufactured by Life Technology)	382 g
Tris (manufactured by Life Technology)	12.1 g
Triton-X 100 (manufactured by ICN)	10 g
Distilled water	1,000 ml

[0063]

Adsorption buffer (Present Invention)

Guanidine hydrochloride (manufactured by Life Technology)	382 g
Tris (manufactured by Life Technology)	12.1 g
Triton-X 100 (manufactured by ICN)	10 g
Acetylene glycol (manufactured by Air Products)	10 g
Silicone oil (manufactured by GE Toshiba Silicone)	2 g
Distilled water	1,000 ml

[0064]

Washing Buffer	
10 mmol/L Tris-HCl	65% ethanol

[0065]

(2) Operation for purifying nucleic acid

To human whole blood sample (200 µl) were added 200 µl of the adsorption buffer (2 types from Comparative Example and the present invention) and 200 µl of protease K and incubation was conducted at 60°C for 10 minutes. After the incubation, 200 µl of ethanol was added followed by stirring. After the stirring, the solution was injected into to a cartridge for purifying nucleic acid having a structure as shown in Fig. 1 to Fig. 6. After the injection, a liquid was pushed out using a piston.

[0066]

After that, 500 µl of a washing buffer was injected and the liquid was pushed out using a piston to wash the cartridge and impurities on the adsorbed solid phase. Finally, 200 µl of distilled water was injected, the solution was pushed out using a piston and the solution was recovered as a DNA solution.

[0067]

(3) Quantification of recovered amount of nucleic acid

Yields of DNA purified by the operation of (2) and foam height (value where, upon discharging the sample

solution, length of foams generated from the opening was measured) are shown in the following Table 1. From the result of Table 1, it was found that, according to the present invention, generated amount of foams was able to be suppressed.

[0068]

Table 1:

Sample Nos.	DNA (μg)	Height of Foams
1 (Present Invention)	4.2	7 mm
2 (Present Invention)	3.6	5 mm
3 (Present Invention)	5.7	3 mm
4 (Present Invention)	6.3	5 mm
5 (Present Invention)	5.0	11 mm
1 (Comparative Example)	4.4	32 mm
2 (Comparative Example)	3.9	30 mm
3 (Comparative Example)	4.2	24 mm
4 (Comparative Example)	5.8	45 mm
5 (Comparative Example)	6.3	60 mm

[Brief Description of the Drawings]

[0069]

[Fig. 1] Fig. 1 is a sectional side view showing an apparatus for isolating and purifying nucleic acid of the present invention, and an enlarged view showing a part of a piston material with a check valve and a pressure sensor.

[Fig. 2] Fig. 2 is an exploded perspective view showing an apparatus for isolating and purifying nucleic acid of the present invention.

[Fig. 3] Fig. 3 is a sectional side view showing another embodiment of a method for connecting a syringe and

a solid retention material.

[Fig. 4] Fig. 4 is an enlarged sectional view showing a solid retention material.

[Fig. 5] Fig. 5 is a sectional view showing a tip of a syringe.

[Fig. 6] Fig. 6 is an explanatory view showing a flow of liquid pushed out from a receiving part to outside, in a tip of the syringe.

[Fig. 7] Fig. 7 is an explanatory view showing another embodiment of a check valve in a piston material.

[Description of Reference Numerals and Signs]

[0070]

1: APPARATUS FOR ISOLATING AND PURIFYING NUCLEIC ACID

3: SYRINGE

5: PISTON MATERIAL

7: SOLID RETENTION MATERIAL

9: TIP OF SYRINGE

11: FIRST OPENING PART

13: FLANGE PART

15: BASE OF SYRINGE

17: SECOND OPENING PART

18: FIXING MECHANISM

19: RECEIVING PART

21: TAPER

23: MALE SCREW

25: FEMALE SCREW

27: LIQUID GUIDE FACE

29: END OF TIP

31: PLUNGER

33: O-RING

34: OPERATING PART

35: MAIN BODY PART

37: END PLATE

39: NOZZLE

41: PATHWAY HOLE

43: SOLID PHASE SUPPORTING FACE

45: SOLID PHASE

46: POLYPROPYLENE SINTERED FILTER

47: TAPER

49: NARROWED PART

51: CHECK VALVE

53: COMMUNICATING ROUTE

55: VALVE SEAT

57: VALVE PLUG

59: PRESSURE SENSOR

[Designation of Document] ABSTRACT

[Abstract]

[Task] To provide a method for isolating and purifying a nucleic acid, comprising adsorption of nucleic acid onto a solid phase, washing the solid phase under such a state that nucleic acid is adsorbed therewith and desorbing nucleic acid from the solid phase, in which foams generated during the steps are able to be eliminated..

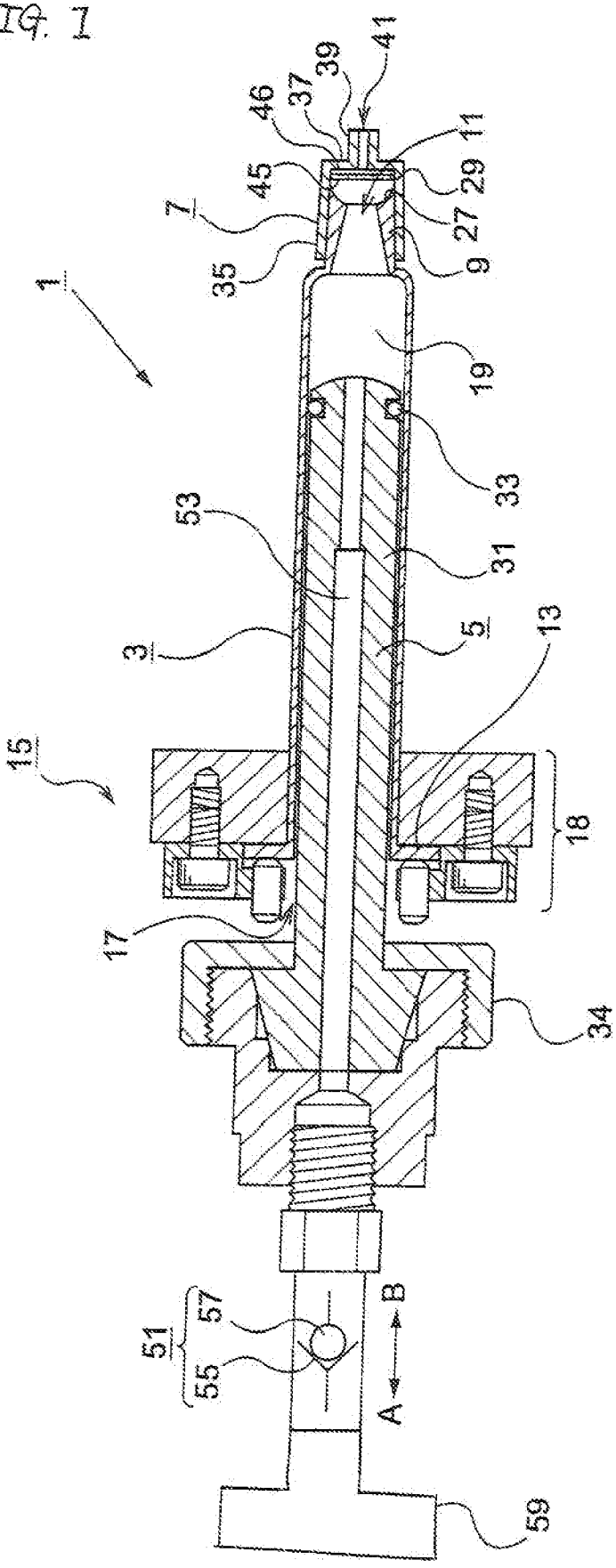
[Means for Resolution] A method for isolating and purifying a nucleic acid, including the steps of adsorbing nucleic acid onto a solid phase, and desorbing nucleic acid from the solid phase, using a solution capable of adsorbing nucleic acid onto a solid phase and a solution capable of desorbing nucleic acid from a solid phase, respectively, in which the solution capable of adsorbing nucleic acid contains an antifoaming agent.

[Selected Drawing] None

【書類名】図面
【図1】

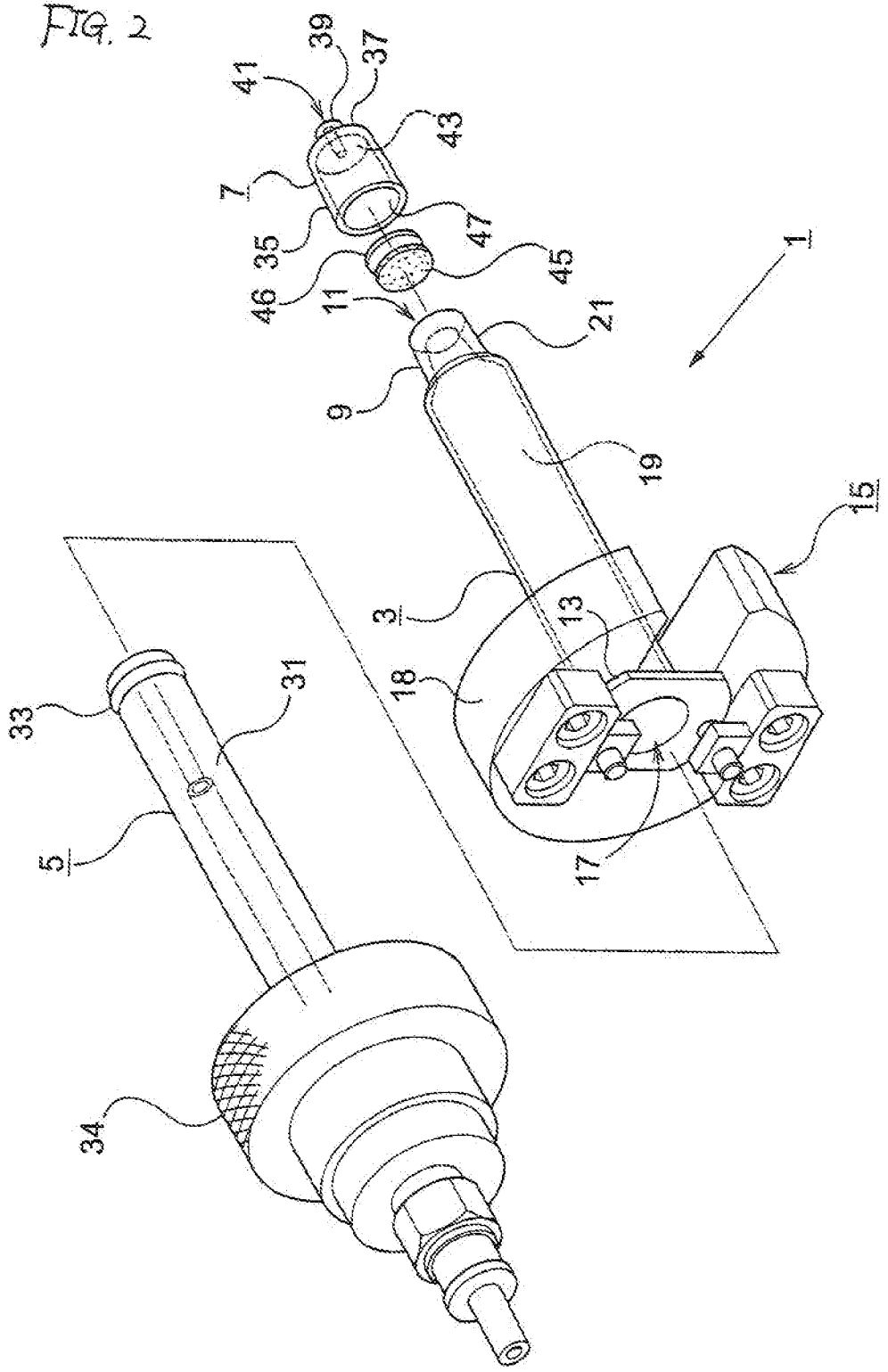
Drawings

FIG. 1



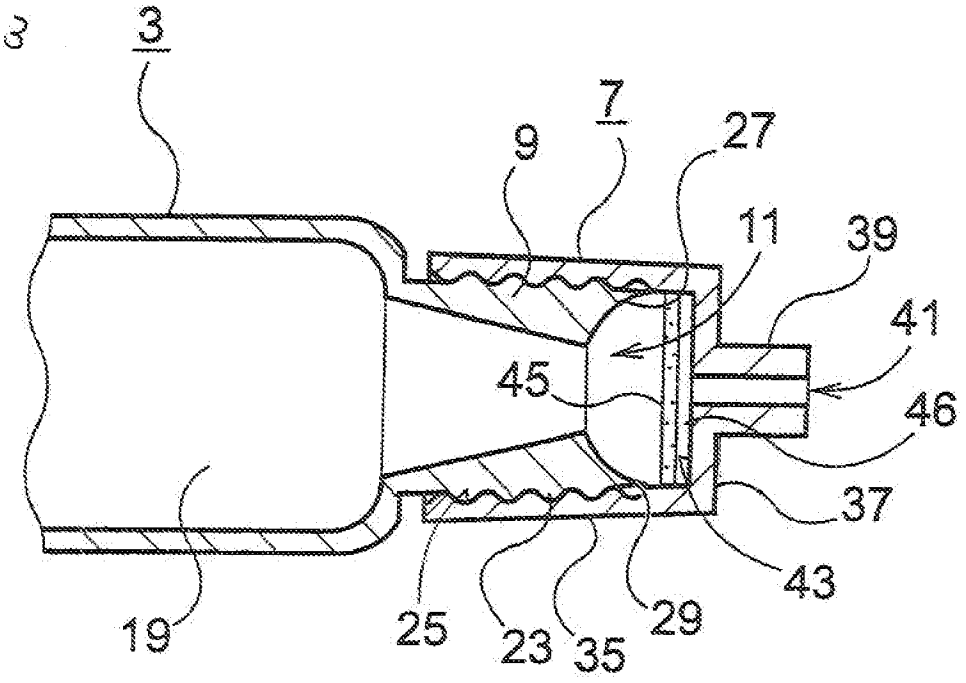
{図2}

FIG. 2



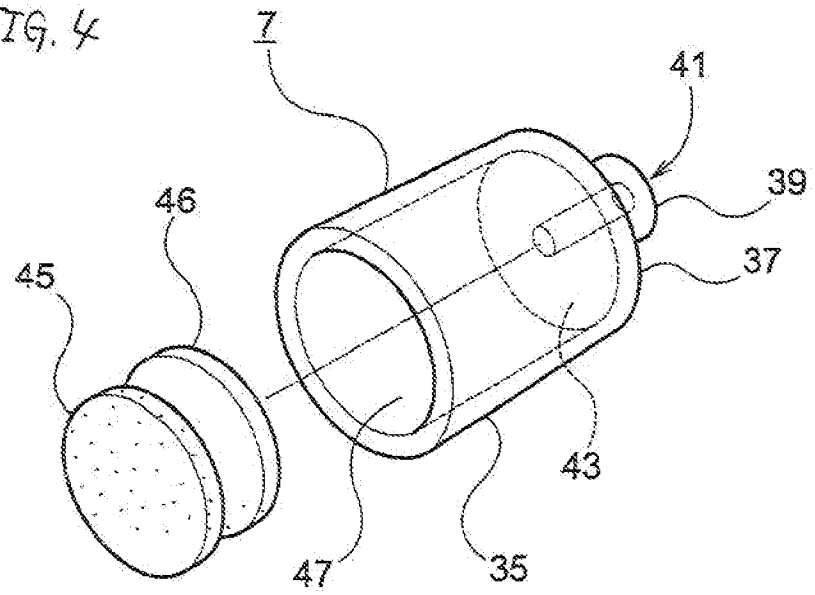
【図3】

FIG. 3



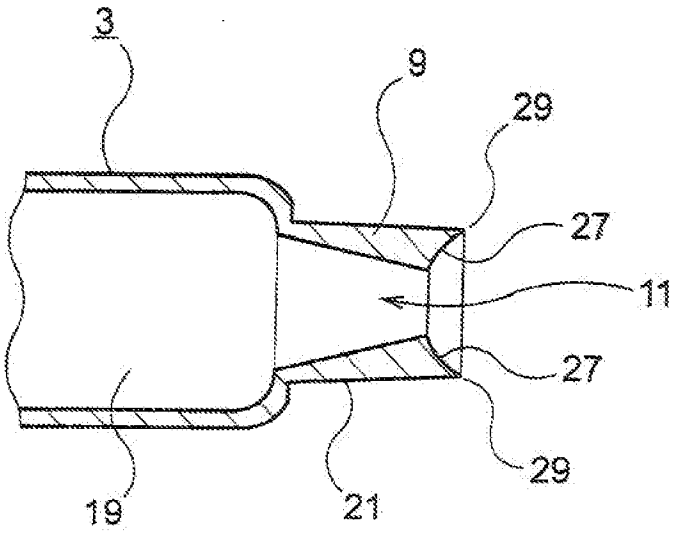
【図4】

FIG. 4



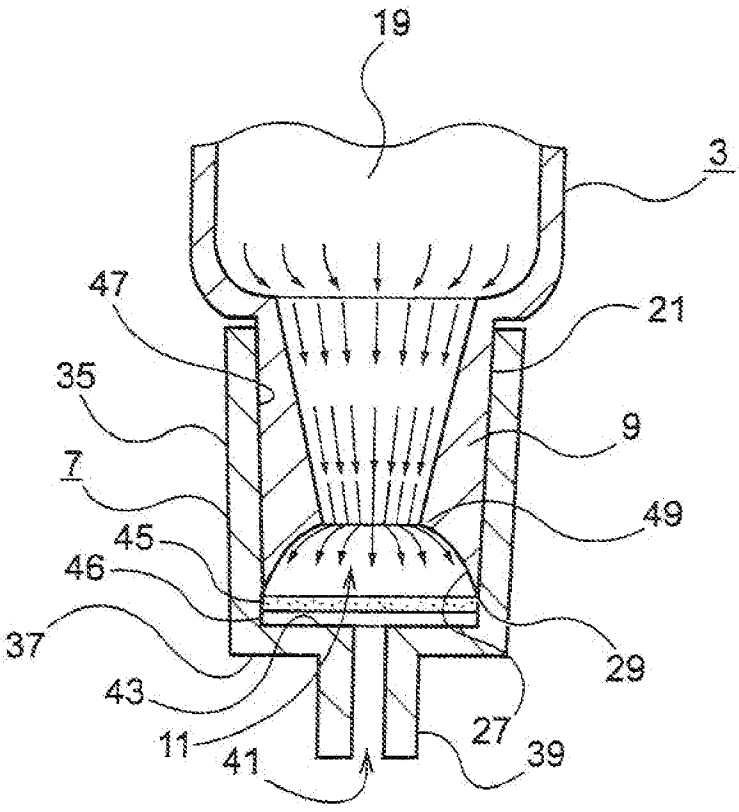
【図5】

FIG. 5



【図6】

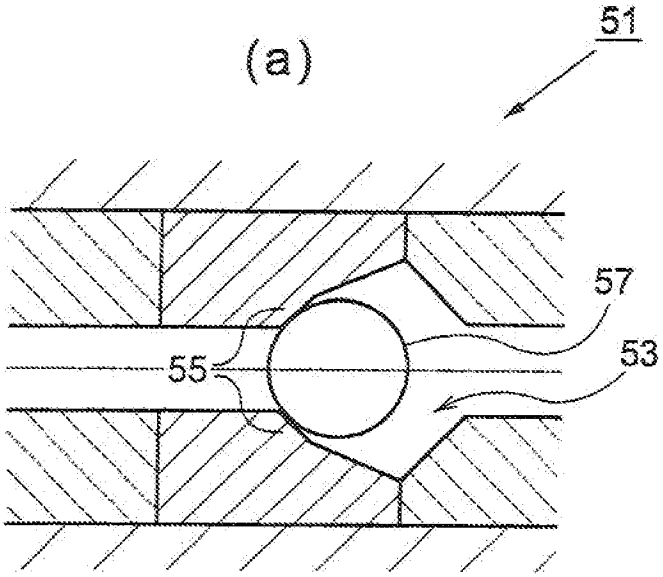
FIG. 6



【図7】

FIG. 7

(a)



(b)